IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:	
AKHVERDIAN et al.	Art Unit: 1652
Application No.: 10/673,786	Examiner: RAMIREZ, Delia M.
Filing Date: September 30, 2003	Attorney Ref. No.: US-115
For: METHOD FOR PRODUCING L-THREONINE USING BACTERIA BELONGING TO THE GENUS ESCHERICHIA	Confirmation No.: 7880

REVISED BRIEF FOR APPELLANT

Mail Stop Appeal Brief - Patents
Commissioner for Patents
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Sir:

COMES NOW the Appellant to present this Brief in support of the appeal of the final rejections of Claims 12, 15-16, 19 and 21-24 in the above-captioned patent application. The Notice of Appeal having been timely filed on May 18, 2007, and a Petition with a one-month extension of time filed on May 18, 2007, this Brief is due to be filed on July 18, 2007.

It is not believed that extensions of time are required, beyond those that may otherwise be provided for in accompanying documents. If, however, additional extensions of time are necessary to prevent abandonment of this application or dismissal of this appeal, then such extensions of time are hereby petitioned under 37 C.F.R. § 1.136(a), and the Commissioner is hereby authorized to charge fees necessitated by this paper, and to credit all refunds and overpayments, to the credit card identified in EFS-Web processing.

For the following reasons, Appellant respectfully submits that the final rejection of each of Claims 12, 15-16, 19 and 23 in this application is in error, and therefore respectfully requests reversal of the rejections.

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I. Real Party in Interest

The real party in interest is Ajinomoto Co., Inc, a corporation of Japan.

II. Related Appeals and Interferences

There are no related appeals or interferences.

III. Status of Claims

Claims 1-11, 13-14, 17-18, 20-22, and 24 are canceled. Claims 12, 15-16, 19 and 23 are pending. No claims are in condition for allowance. Claims 12, 15-16, 19 and 23 stand finally rejected in the Advisory Action dated May 11, 2007, and are on appeal.

IV. Status of Amendments

All amendments to the claims have been entered, including the amendment after final filed on April 18, 2007.

V. Summary of Claimed Subject Matter

The invention is directed a method for producing L- threonine comprising cultivating in a culture medium an L-threonine-producing *Escherichia coli* bacterium and collecting the L-threonine from the culture medium (see paragraph [0053]), wherein the bacterium has been modified to increase the expression of:

i) the aspartate aminotransferase gene encoding the protein comprising the amino acid sequence of SEQ ID NO. 2 (see paragraph [0034]),

ii) the *Escherichia coli thrA* gene which codes for an aspartokinase homoserine dehydrogenase I which is resistant to feedback inhibition by threonine (see paragraph [0051]),

iii) the *Escherichia coli thrB* gene (see paragraph [0051]),

iv) the *Escherichia coli thrC* gene (see paragraph [0051]), and

v) the *Escherichia coli rhtA* gene (see paragraph [0052]),

wherein the expression of the genes is increased by a method selected from the group

consisting of increasing the copy number of said genes (see paragraph [0041]) and placing said genes under the control of a potent promoter (see paragraph [0043]).

VI. Ground of Rejection to Be Reviewed on Appeal

Whether Claims 12, 15-16, 19, and 23 are unpatentable under 35 U.S.C. §103 over the disclosure of Katsumata et al. in view of the disclosures of Debabov et al., Edwards et al., and further in view of Kishino et al..

VII. Argument

In the Final Rejection dated January 18, 2007, beginning at page 5, Claims 12, 15-16, 19, and 23 were rejected under 35 U.S.C. § 103 over Katsumata et al. in view of the disclosure of Debabov et al., Edwards et al., and further in view of Kishino et al. This rejection was upheld in the Advisory Action issued on May 11, 2007. For at least the following reasons, this rejection is in error and should be reversed.

A. Legal Standard

Claimed subject matter is obvious in light of the prior art if it would have been obvious to one of ordinary skill in the relevant art at the time the invention was made. 35 U.S.C. § 103(a). In considering the entire prior art in the relevant field, the claimed subject matter is obvious if the prior art "would have suggested to one of ordinary skill in the art that this [invention should be made] and would have a reasonable likelihood of success." *In re Dow Chemical Co.*, 837 F.2d 469, 473 (Fed. Cir. 1988).

Obviousness can be shown either directly by demonstrating the technical motivation to combine the prior art, *Life Technologies, Inc. v. Clontech Laboratories, Inc.*, 224 F.3d 1320, 1326 (Fed. Cir. 2000), or indirectly through "secondary considerations" after the claimed subject matter was invented, *Custom Accessories, Inc. v. Jeffrey-Allan Industries, Inc.*, 807 F.2d 955, 960 (Fed. Cir. 1986). To show the motivation to combine prior art, it is not enough to simply identify different references that might be combined in hindsight; showing obviousness requires

showing a motivation to combine the pieces. *Velander v. Garner*, 348 F.3d 1359, 1363 (Fed. Cir. 2003). That motivation might come from a reference or from the knowledge of an artisan of ordinary skill. The level of ordinary skill in an art is based on a number of factors, including the educational level of the inventor, the type of problems encountered in the art, prior solutions to those problems, and the speed of innovation in the art. *Ruiz v. A.B. Chance Co.*, 234 F.3d 654, 666-67 (Fed. Cir. 2000).

"Secondary considerations" focus on how the invention was received in the market; a very successful or surprising invention is probably not obvious. *See Custom Accessories, Inc.*, 807 F.2d at 960. The "secondary considerations" considered by the courts include: commercial success, copying of the invention by others, licensing of the invention, evidence of a long-felt need for the invention, skepticism by skilled artisans that the claimed invention could be achieved, prior failures of others to achieve the same result, and unexpected results. *Id.*; *Pentec, Inc. v. Graphic Controls Corp.*, 776 F.2d 309, 316 (Fed. Cir. 1985). No secondary consideration is required for an invention to be non-obvious, but a court may use evidence of secondary considerations in its determination of obviousness (or non-obviousness). *Custom Accessories, Inc.*, 807 F.2d at 960.

Finally, the "teaching, suggestion, motivation" test (the "TSM" test) which is often used to prove obviousness was revised somewhat in a recent Supreme Court decision. See *KSR International Co. v. Teleflex Inc. et al.,* No. 04-1350, slip op. at 16 (S.Ct., April 30, 2007). This test, as applied in this case, was determined to be too rigidly applied. The Supreme Court said: "There is no necessary inconsistency between the idea underlying the TSM test and the Graham analysis. But when a court transforms the general principle into a rigid rule that limits the obviousness inquiry . . . it errs." The Federal Circuit erred by (1) looking only at the problem the patentee was trying to solve, (2) assuming that a person of ordinary skill attempting to solve a problem will be led only to those elements of prior art designed to solve the same problem, (3) concluding that a patent claim cannot be proved obvious by showing the combination of elements was "obvious to try," and (4) applying a rigid rule to prevent hindsight that denied factfinders "recourse to common sense."

In its broadest sense, the *KSR* decision broadens the inquiry that must be made when proving an invention is obvious over prior art. Before this decision, when combining references, an Examiner must show a motivation by the person of skill in the art to combine the teachings of the different references. In the decision, the Supreme Court states that such a showing is not required, but only a "reason" to combine the teachings should be shown. This reason can be based upon logic, common sense, and/or the knowledge in the art. The Court stated that when familiar elements are combined according to known methods, the invention is likely to be obvious when it does no more than yield predictable results." *KSR, slip op.* at 12.

Also, the Court explained that when the prior art elements work together in an unexpected and fruitful manner, the invention is not obvious. *Id.* (*citing United States v. Adams,* 383 U.S. 39, 40 (1966)). If, however, the combination of old elements does no more than they would in separate, sequential operation, even though the combination might perform a useful function, the combination is likely obvious. *Id.* at 13 (*citing Anderson's-Black Rock, Inc. v. Pavement Salvage Co.,* 396 U.S. 57 (1969)).

These statements regarding expectation are not so different from the current test for proving obviousness, however, the Court does broaden the analysis in its discussion of expanding of the "teaching, suggestion, or motivation" test. As stated above, a specific or rigid motivation is no longer required, but only a logical reason for combining prior art. This standard it less stringent.

B. The rejection of Claims 12, 15-16, 19 and 23 under 35 U.S.C. §103 is in error

In the January 18, 2007 Final Office Action, claims 12, 15-16, 19, and 23 were rejected under 35 U.S.C. §103 over Katsumata et al. in view of the disclosures of Debabov et al., Edwards et al., and further in view of Kishino et al..

The present invention relates to a method for producing L-threonine comprising cultivating an L-threonine producing *E. coli* which has been modified to enhance the activity of aspartate aminotransferase, otherwise known as AspC, having the amino acid sequence of SEQ ID NO: 2, as well as enhancing the expression of the following known genes: a feedback-

resistant *thrA, thrB, thrC, and rhtA.* All of the genes are derived from *Escherichia coli.*.

Many types of aminotransferase enzymes are known, and their substrate specificities are each different. For example, it is described in the left column of the page 7639, J. Bacteriol., 187, 7639-7646 (2005) [EXHIBIT A, submitted with the response filed October 19, 2006 and attached hereto], that 16 types of aminotransferases exist in *E. coli*, and 14 types in *Corynebacterium glutamicum*. These aminotransferases are involved in the synthesis of many kinds of amino acids. It is not clear from the prior art, however, which type of aminotransferase is effective for increasing a production of L-threonine.

Katsumata et al. disclose L-threonine production using an aminotransferase gene cloned from *Corynebacterium glutamicum;* however, it is clear that the disclosed gene is not the aspartate aminotransferase depicted in SEQ ID NO. 2. In fact, it is not clear what type of aminotransferase is encoded by the prior art gene. As the Office Action acknowledges, Katsumata et al. does not teach production of L-threonine, nor the aspartate aminotransferase of SEQ ID NO. 2.

Debabov et al., Edwards et al., and Kishino et al. fail to make up for the deficiencies of Katsumata et al.. Debabov et al. also fails to teach the aspartate aminotransferase of SEQ ID NO. 2, but teach increased expression of thrA, thrB, thrC, and rhtA. Since there is no disclosure of the aspartate amiontransferase of SEQ ID NO. 2, this reference fails to make up for the deficiency of Katsumata et al.

Edwards et al. is cited for disclosing that aspartate aminotransferase is effective for L-phenylalanine production in *E. coli*. However, the L-threonine biosynthesis pathway is completely different from the L-phenylalanine biosynthesis pathway. Namely, L-phenylalanine is generated from phenyl pyruvate acid by transamination catalyzed by aminotransferase, whereas L-threonine is not generated by direct transamination catalyzed by aminotransferase. From this teaching, one of ordinary skill in the art would not have been able to determine or deduce that increasing the expression of the aspartate aminotransferase gene of *E. coli* would be effective for producing L-threonine, since the production pathway of L-phenylalanine is completely different from that of L-threonine. Therefore, Edwards et al. fails to make up for the

deficiencies of Katsumata et al..

Kishino et al. teach the use of low copy vectors in preferred strains of *E.coli* for L-threonine production; however, they fail to teach the method of increasing expression of the gene of SEQ ID NO. 1 to increase L-threonine production. Therefore, Kishino et al. fail to make up for the deficiencies of Katsumata et al..

Neither the primary reference of Katsumata et al., nor any of the secondary references, teach L-threonine production in *E. coli* which have been modified to have increased gene expression of the aspartate aminotransferase gene, depicted in SEQ ID NO. 1 and encoding a protein depicted in SEQ ID NO. 2. Furthermore, no combination of the teachings would suggest such a teaching. As the main feature of the claimed invention, the connection between the increase in gene expression of the gene encoding aspartate aminotransferase of SEQ ID NO.2 and the increased production of L-threonine, is not remotely suggested by the cited references, either singly or in any combination.

On page 6 of the Office Action issued January 18, 2007, it is argued that Katsumata teach a protein having the same enzymatic activity as the polypeptide of SEQ ID NO: 2, and the increase in L-threonine production as a result of increasing the synthesis of an enzyme from *C. glutamicum* having aspartate aminotransferase activity. Furthermore, it is argued that Debabov et al. is a high L-threonine producer which has been modified to increase expression of the *thrA, thrB, thrC, and rhtA* genes.

It would not have been expected by the ordinarily skilled art worker that an additional increase in threonine production would result by increasing the expression of the gene depicted in SEQ ID NO: 2, because threonine production is *already* optimized at a high level in a bacterium with increased expression of the *thrA*, *thrB*, *thrC*, and *rhtA* genes. Furthermore, it is acknowledged (and pointed out in the January 18, 2007 Office Action on page 6) that the *aspC* gene (SEQ ID NO: 2) encodes an enzyme which catalyzes the synthesis of aspartic acid from oxaloacetic acid. However, one of ordinary skill in the art would know that even if expression of the *aspC* gene is increased, the production of threonine would not also increase unless the supply of aspartic acid runs short in the whole pathway of threonine synthesis. The shortage of aspartic

acid will occur if the synthesis reaction of aspartic acid from oxaloacetic acid is the rate-limiting step. However, one of ordinary skill in the art would not have known which reaction is the rate-limiting step in threonine synthesis. Therefore, the additional increase in threonine as a result of the combination of increasing expression of the *aspC* gene with increasing expression of the *thrA*, *thrB*, *thrC* and *rhtA* genes is completely unexpected.

Thus, one of ordinary skill in the art would know that the achieving increased threonine production will not occur merely by increasing the expression of *aspC* in E. coli which has increased expression of *thrA*, *thrB*, *thrC*, and *rhtA*. Therefore, the result of the present claimed method is completely unexpected, and is a novel and unobvious result.

Turning to the Advisory Action dated May 11, 2007, it is stated on page 2 that "[t]here is no evidence in the specification or the art that the maximum amount of L-threonine that can be produced in *E.coli* is that obtained from an *E.coli* which has increased expression of the thrA, thrB, thrC, and rhtA genes". First, the claims do not require the 'maximum amount' of L-threonine to be produced. Furthermore, the specification clearly describes in paragraph [0005] the threonine producing strain B-3996, which is known to be the best threonine producer at the time of filing of the instant application, and which has enhanced expression of the thrA, thrB, thrC, and rhtA genes.

On page 3-4 of the Advisory Action, it is stated that "it is clear from the art that there is not only motivation but a reasonable expectation of success at increasing production of L-threonine by increasing the expression of the aspC gene." The Action further references paragraph [0010] of the specification and the art cited therein as teaching "that a similar approach (increased expression of the aspC gene) was followed in the production of L-lysine in *E.coli*, which is also synthesized from aspartic acid, that resulted in increased production of L-lysine". This assessment is simply incorrect. The art cited in the specification which is referred to in the Advisory Action (US Patent 6,040,160 – provided as Exhibit B as evidence relied upon by the Examiner in any ground of rejection to be reviewed upon appeal, 37 C.F.R. 41.67(c)(1)(ix)) contains tables 8, 10, 12, 14, 18, 19, 20, and 21, which show the effect of increasing expression of various genes on the production of L-lysine. In these tables, "paspC"

indicates a plasmid containing the aspC gene. It can be seen from these tables that the production of L-lysine is hardly increased in all strains transformed with the paspC. It is clear from these data that increasing the expression of the aspC gene does not lead to increased L-lysine production in a strain wherein the supply of aspartic acid is not rate-limiting for L-lysine production. Similarly, in the production of threonine in *E.coli*, increasing the expression of the aspC gene does not lead to increased threonine production unless the supply of aspartic acid is rate-limiting. Therefore, since the threonine synthetic pathway is very complex and the rate-limiting step is unknown, it would not have been obvious to one of ordinary skill in the art that increasing the aspC expression would lead to increased threonine production. The present invention is the first to show that threonine production is increased by combining increased expression of the aspC, thrA, thrB, thrC, and rhtA genes all together, which is clearly not expected by the combination of the cited prior art.

For at least the foregoing reasons, Appellants respectfully submit that the subject matters of the Claims, each taken as a whole, would not have been obvious to one of ordinary skill in the art at the time of Appellant's invention, are therefore not unpatentable under 35 U.S.C. § 103(a), and therefore respectfully request withdrawal of the rejection thereof under 35 U.S.C. § 103(a).

IX. Conclusion

For at least the foregoing reasons, Appellant respectfully submits that the subject matters of Claims 12, 15-16, 19 and 23, each taken as a whole, are patentable. Accordingly, Appellant respectfully requests reversal of the rejections of Claims 12, 15-16, 19 and 23 under section 103.

Respectfully submitted,

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APPENDIX A: CLAIMS ON APPEAL

12. A method for producing L- threonine comprising:

A) cultivating in a culture medium an L-threonine-producing *Escherichia coli* bacterium, wherein the bacterium has been modified to increase the expression of:

i) the aspartate aminotransferase gene encoding the protein comprising the amino acid sequence of SEQ ID NO. 2,

ii) the *Escherichia coli thrA* gene which codes for an aspartokinase homoserine dehydrogenase I which is resistant to feedback inhibition by threonine,

iii) the *Escherichia coli thrB* gene,

iv) the *Escherichia coli thrC* gene, and

v) the *Escherichia coli rhtA* gene,

wherein said expression of said genes is increased by a method selected from the group consisting of increasing the copy number of said gene and placing said gene under the control of a potent promoter, and

B) collecting the L-threonine from the culture medium.

15. The method according to claim 12, wherein said expression of the aspartate aminotransferase gene is increased by increasing the copy number of the aspartate aminotransferase gene.

16. The method of claim 15, wherein the copy number is increased by transforming said bacterium with a low copy number vector containing said gene.

19. The method of claim 12, wherein said aspartate aminotransferase gene comprises a DNA comprising the nucleotides 1 to 1191 in SEQ ID NO: 1.

23. The method according to claim 12, wherein the potent promoter is selected from the

group consisting of the lac promoter, trp promoter, trc promoter, PR promoter, and PL promoter.

APPENDIX B: EVIDENCE

Exhibit A: Marienhagen, et al., J. Bacteriol., 187, 7639-7646 (2005), particularly page 7639, submitted with the response filed October 19, 2006.

Exhibit B: US Patent 6,040,160 – provided as evidence relied upon by the Examiner in any ground of rejection to be reviewed upon appeal, see 37 C.F.R. 41.67(c)(1)(ix), cited to by the Examiner in the Advisory Action dated May 11, 2007.

APPENDIX C: RELATED PROCEEDINGS

None.